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RESEARCH ARTICLE

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# Caulicolous *Botryosphaeriales* from Thailand

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**Key words**

*Aplosporella*
*Botryosphaeriaceae*
*Diplodia*
*Lasiodiplodia*
multigene phylogeny
*Pseudofusicoccum*
sexual morph
systematics

**Abstract** Members of *Botryosphaeriales* are commonly encountered as endophytes or pathogens of various plant hosts. The *Botryosphaeriaceae* represents the predominant family within this order, containing numerous species associated with canker and dieback disease on a wide range of woody hosts. During the course of routine surveys from various plant hosts in Thailand, numerous isolates of *Botryosphaeriaceae*, including *Aplosporellaceae* were collected. Isolates were subsequently identified based on a combination of morphological characteristics and phylogenetic analysis of a combined dataset of the ITS and EF1-α gene regions. The resulting phylogenetic tree revealed 11 well-supported clades, correlating with different members of *Botryosphaeriales*. Other than confirming the presence of taxa such as *Lasiodiplodia theobromae*, *L. pseudotheobromae* and *Neofusicoccum parvum*, new records for Thailand include *Pseudofusicoccum adansoniae* and *P. ardesiacum*. Furthermore, four novel species are described, namely *Diplodia neojuniperi* from *Juniperus chinensis*, *Lasiodiplodia thailandica* from *Mangifera indica*, *Pseudofusicoccum artocarpi* and *Aplosporella artocarpi* from *Artocarpus heterophyllus*, while a sexual morph is also newly reported for *L. gonubiensis*. Further research is presently underway to determine the pathogenicity and relative importance of these species on different woody hosts in Thailand.

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## INTRODUCTION

The *Botryosphaeriaceae* was introduced as family in the *Botryosphaeriales* by Schoch et al. (2006). Based on recent molecular phylogenetic studies, however, several species have been excluded from the *Botryosphaeriaceae* and allocated to different families within the order, namely *Planistromellaceae* (*Kellermania*) (Minnis et al. 2012), *Phyllostictaceae* (*Phyllosticta*) (Wikee et al. 2013), *Aplosporellaceae* (*Aplosporella* and *Bagnisiella*), *Saccharataceae* (*Saccharata*) and *Melanopsaceae* (*Melanops*) (Slippers et al. 2013). The *Botryosphaeriaceae* represents the predominant family of this order, and Phillips et al. (2013) provided phylogenetic support for 17 genera including *Barriopsis*, *Botryobambusa*, *Botryosphaeria*, *Cophinforma*, *Diplodia*, *Dothiorella*, *Endomelanconiopsis*, *Lasiodiplodia*, *Macrophomina*, *Neodeightonia*, *Neofusicoccum*, *Neoscytalidium*, *Phaeobotryon*, *Pseudofusicoccum*, *Spencermartinsia*, *Sphaeropsis* and *Tiarosporella*. Species of *Botryosphaeriaceae* have a cosmopolitan distribution on a wide range of plant hosts, encompassing endophytes, saprobes and plant pathogens (von Arx & Müller 1954, Slippers & Wingfield 2007). Recent studies have revealed that some of them are severe canker and dieback pathogens of a range of important crops such as *Proteaceae* cut-flowers (Denman et al. 2003, Marincowitz et al. 2008), *Eucalyptus* (Slippers et al. 2004b, 2007, Zhou et al. 2008), grapevines (van Niekerk et al. 2006, Urbez-Torres et al. 2012), oaks (Sánchez et al. 2003), pines (Mohali et al. 2007) and stone fruits (Damm et al. 2007a, Slippers et al. 2007, Quaglia et al. 2014). Furthermore, these fungi also cause fruit diseases, which are mainly associated with fruit and stem-end rot as reported in avocado (McDonald & Eskalen 2011), mango (Ismail et al. 2012, Marques et al. 2013) and olives (Lazzizera et al. 2008).

Members of the *Botryosphaeriaceae* are known to be widely distributed, occurring on a broad range of plant hosts in many countries, including Thailand (Trakunyingcharoen et al. 2014). Liu et al. (2012) accepted 29 genera in the *Botryosphaeriales*, reported six new species from Thailand, and introduced two new genera, namely *Botryobambusa* (*B. fusicoccum*) and *Cophinforma* (*C. eucalypti*). Furthermore, four new species were described, namely *Auerswaldia dothiorella* (= *Dothiorella thailandica*), *A. lignicola* (= *Lasiodiplodia lignicola*), *Botryosphaeria fusispora* and *Phaeobotryosphaeria eucalypti* (see Phillips et al. 2013). Other records for Thailand included *Botryosphaeria agaves*, *Lasiodiplodia theobromae*, *Neodeightonia subglobosa* and *Neofusicoccum parvum* (Liu et al. 2012). In addition, *Lasiodiplodia pseudotheobromae* was also newly associated with mango diseases in this country (Trakunyingcharoen et al. 2013).

Until relatively recently, species of *Botryosphaeriaceae* have been identified solely based on morphological characteristics (Denman et al. 2000, Xenopoulos & Tsopelas 2000). However, since conidial septation and pigmentation evolved more than once within different genera of the family (Slippers et al. 2013) and are strongly influenced by cultural conditions (Alves et al. 2006), misidentifications have proven to be rather common in the literature. In this regard, molecular phylogenetic studies have provided a powerful tool to accurately identify members of *Botryosphaeriaceae* based on a combination of different partial gene regions, including β-tubulin (TUB), translation elongation factor1-α (EF1-α), the internal transcribed spacers (ITS) of the nrDNA, and the small and large-subunit ribosomal rRNA genes (SSU and LSU) (Slippers et al. 2004a, 2005, 2013, Crous et al. 2006).

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**Table 1** Details and GenBank accession numbers of isolates of *Botryosphaeriaceae* included in this study. New isolates obtained in this study are indicated in **bold**, new GenBank sequence accession numbers in *italics*, and * represents ex-type isolates.

| Species | Accession no.[1] | Substrate | Locality | Collector | GenBank[2] | |
|---|---|---|---|---|---|---|
| | | | | | ITS | EF1-α |
| *Aplosporella artocarpi* | **CPC 22791** | *Artocarpus heterophyllus* | Thailand | T. Trakunyingcharoen | *KM006450* | *KM006481* |
| *A. prunicola* | CBS 121167* | *Prunus persica* | South Africa | U. Damm | EF564376 | – |
| *A. yalgorensis* | MUCC 511* | *Acacia cochlearis* | Australia: Western Australia | K.M. Taylor | EF591926 | EF591977 |
| *Barriopsis fusca* | CBS 174.26* | *Citrus* sp. | Cuba | N.E. Stevens | EU673330 | EU673296 |
| *Ba. iraniana* | CBS 124698* | *Mangifera indica* | Iran | J. Abdollahzadeh & A. Javadi | FJ919663 | FJ919652 |
| *Botryosphaeria dothidea* | CBS 115476* | *Prunus* sp. | Switzerland | B. Slippers | AY236949 | AY236898 |
| *Bo. fabicerciana* | CBS 127193* | *Eucalyptus* sp. | China | M.J. Wingfield | HQ332197 | HQ332213 |
| *Bo. ramosa* | CBS 122069* | *Eucalyptus camaldulensis* | Australia: Western Australia | T.I. Burgess | EU144055 | EU144070 |
| *Botryosphaeria* sp. | **CPC 22789** | *Bouea burmaxnica* | Thailand | T. Trakunyingcharoen | *KM006448* | *KM006479* |
| *Diplodia africana* | CBS 120835* | *Prunus persica* | South Africa | U. Damm | EF445343 | EF445382 |
| *Di. agrifolia* | CBS 132777* | *Quercus agrifolia* | USA | S. Lynch & A. Eskalen | JN693507 | JQ517317 |
| *Di. bulgarica* | CBS 124254* | *Malus sylvestris* | Bulgaria | S.G. Bobev | GQ923853 | GQ923821 |
| *Di. corticola* | CBS 112549* | *Quercus suber* | Portugal | A. Alves | AY259100 | AY573227 |
| *Di. cupressi* | CBS 168.87* | *Cupressus sempervirens* | Israel | Z. Solel | DQ458893 | DQ458878 |
| *Di. malorum* | CBS 124130* | *Malus sylvestris* | Portugal | A.J.L. Phillips | GQ923865 | GQ923833 |
| *Di. mutila* | CBS 112553 | *Vitis vinifera* | Portugal | A.J.L. Phillips | AY259093 | AY573219 |
| *Di. neojuniperi* | **CPC 22753*** | *Juniperus chinensis* | Thailand | T. Trakunyingcharoen | *KM006431* | *KM006462* |
| | **CPC 22754** | *Juniperus chinensis* | Thailand | T. Trakunyingcharoen | *KM006432* | *KM006463* |
| | **CPC 22802** | *Juniperus chinensis* | Thailand | T. Trakunyingcharoen | *KM006457* | *KM006488* |
| *Di. olivarum* | CBS 121887* | *Olea europaea* | Italy | S. Frisullo | EU392302 | EU392279 |
| *Di. pseudoseriata* | CBS 124906* | *Blepharocalyx salicifolius* | Uruguay | C. Perez | EU080927 | EU863181 |
| *Di. quercivora* | CBS 133852* | *Quercus canariensis* | Tunisia | B.T. Linaldeddu | JX894205 | JX894229 |
| *Di. rosulata* | CBS 116470* | *Prunus africana* | Ethiopia | A. Gure | EU430265 | EU430267 |
| *Di. sapinea* | CBS 109726 | *Pinus patula* | Indonesia | M.J. Wingfield | DQ458896 | DQ458881 |
| | CBS 393.84* | *Pinus nigra* | Netherlands | H.A. van der Aa | DQ458895 | DQ458880 |
| *Di. seriata* | CBS 112555* | *Vitis vinifera* | Portugal | A.J.L. Phillips | AY259094 | AY573220 |
| *Di. tsugae* | CBS 418.64* | *Tsuga heterophylla* | Canada | A. Funk | DQ458888 | DQ458873 |
| *Dothiorella iberica* | CBS 115041* | *Quercus ilex* | Spain | J. Luque | AY573202 | AY573222 |
| *Do. longicollis* | CBS 122068* | *Lysiphyllum cunninghamii* | Australia: Western Australia | T.I. Burgess | EU144054 | EU144069 |
| *Do. thailandica* | CBS 133991* | Dead bamboo culm | Thailand | D.Q. Dai, J.K. Liu & K.D. Hyde | JX646796 | JX646861 |
| *Endomelanconiopsis endophytica* | CBS 120397* | *Theobroma cacao* | Panama | E. Rojas, L. Mejia & Z. Maynard | EU683656 | EU683637 |
| *E. microspora* | CBS 353.97* | Soil | Papua New Guinea | H.A. van der Aa | EU683655 | EU683636 |
| *Lasiodiplodia citricola* | CBS 124707* | *Citrus* sp. | Iran | J. Abdollahzadeh & A. Javadi | GU945354 | GU945340 |
| *L. crassispora* | CBS 118741* | *Eucalyptus urophylla* | Venezuela | S. Mohali | DQ103552 | DQ103559 |
| *L. egyptiacae* | CBS 130992* | *Mangifera indica* | Egypt | A.M. Ismail | JN814397 | JN814424 |
| *L. euphorbicola* | CMM 3609* | *Jatropha curcas* | Brazil | A.R. Machado & O.L. Pereira | KF234543 | KF226689 |
| *L. gilanensis* | CBS 124704* | – | Iran | J. Abdollahzadeh & A. Javadi | GU945351 | GU945342 |
| *L. gonubiensis* | CBS 115812* | *Syzygium cordatum* | South Africa | D. Pavlic | DQ458892 | DQ458877 |
| *L. gonubiensis* (sexual morph) | **CPC 22781** | *Phyllanthus emblica* | Thailand | T. Trakunyingcharoen | *KM006443* | *KM006474* |
| *L. hormozganensis* | CBS 124709* | *Olea* sp. | Iran | J. Abdollahzadeh & A. Javadi | GU945355 | GU945343 |
| *L. iraniensis* | CBS 124710* | *Salvadora persica* | Iran | J. Abdollahzadeh & A. Javadi | GU945348 | GU945336 |
| *L. jatrophicola* | CMM 3610* | *Jatropha curcas* | Brazil | A.R. Machado & O.L. Pereira | KF234544 | KF226690 |
| *L. macrospora* | CMM 3833* | *Jatropha curcas* | Brazil | A.R. Machado & O.L. Pereira | KF234557 | KF226718 |
| *L. mahajangana* | CBS 124927* | *Terminalia catappa* | Madagascar | J. Roux | FJ900597 | FJ900643 |
| *L. margaritacea* | CBS 122519* | *Adansonia gibbosa* | Australia: Western Australia | T.I. Burgess | EU144050 | EU144065 |
| *L. missouriana* | CBS 128311* | *Vitis vinifera* | USA | K. Striegler & G.M. Leavitt | HQ288225 | HQ288267 |
| *L. parva* | CBS 456.78* | Soil from *Cassava*-field | Columbia | O. Rangel | EF622083 | EF622063 |
| *L. plurivora* | CBS 120832* | *Prunus salicina* | South Africa | U. Damm | EF445362 | EF445395 |
| *L. pseudotheobromae* | CBS 116459* | *Gmelina arborea* | Costa Rica | J. Carranza-Velazquez | EF622077 | EF622057 |
| | **CPC 22756** | *Osmanthus fragrans* | Thailand | T. Trakunyingcharoen | *KM006434* | *KM006465* |
| | **CPC 22758** | *Hevea brasiliensis* | Thailand | T. Trakunyingcharoen | KJ607141 | KJ607151 |
| | **CPC 22759** | *Hevea brasiliensis* | Thailand | T. Trakunyingcharoen | KJ607142 | KJ607152 |
| | **CPC 22760** | *Hevea brasiliensis* | Thailand | T. Trakunyingcharoen | KJ607143 | KJ607153 |
| | **CPC 22761** | *Hevea brasiliensis* | Thailand | T. Trakunyingcharoen | KJ607144 | KJ607154 |
| | **CPC 22762** | *Hevea brasiliensis* | Thailand | T. Trakunyingcharoen | KJ607145 | KJ607155 |
| | **CPC 22770** | *Persea americana* | Thailand | T. Trakunyingcharoen | KJ607146 | KJ607156 |
| | **CPC 22771** | *Persea americana* | Thailand | T. Trakunyingcharoen | KJ607147 | KJ607157 |
| | **CPC 22776** | *Psidium* sp. | Thailand | T. Trakunyingcharoen | *KM006438* | *KM006469* |
| | **CPC 22777** | *Coffea arabica* | Thailand | T. Trakunyingcharoen | *KM006439* | *KM006470* |
| | **CPC 22778** | *Psidium* sp. | Thailand | T. Trakunyingcharoen | *KM006440* | *KM006471* |
| | **CPC 22779** | *Dimocarpus longan* | Thailand | T. Trakunyingcharoen | *KM006441* | *KM006472* |
| | **CPC 22783** | *Mangifera indica* | Thailand | T. Trakunyingcharoen | KJ193638 | KJ193682 |
| | **CPC 22784** | *Ficus racemosa* | Thailand | T. Trakunyingcharoen | *KM006444* | *KM006475* |
| | **CPC 22787** | *Mangifera indica* | Thailand | T. Trakunyingcharoen | KJ193639 | KJ193683 |
| | **CPC 22788** | *Bouea burmanica* | Thailand | T. Trakunyingcharoen | *KM006447* | *KM006478* |
| | **CPC 22790** | *Syzygium samarangense* | Thailand | T. Trakunyingcharoen | *KM006449* | *KM006480* |
| | **CPC 22792** | *Phyllanthus acidus* | Thailand | T. Trakunyingcharoen | *KM006451* | *KM006482* |
| | **CPC 22793** | *Mangifera indica* | Thailand | T. Trakunyingcharoen | KJ193640 | KJ193684 |
| | **CPC 22794** | *Mangifera indica* | Thailand | T. Trakunyingcharoen | KJ193641 | KJ193685 |
| | **CPC 22799** | *Cananga odorata* | Thailand | T. Trakunyingcharoen | *KM006455* | *KM006486* |
| | **CPC 22801** | *Dimocarpus longan* | Thailand | T. Trakunyingcharoen | *KM006456* | *KM006487* |
| | **CPC 22803** | *Juniperus chinensis* | Thailand | T. Trakunyingcharoen | *KM006458* | *KM006489* |
| *L. rubropurpurea* | CBS 118740* | *Eucalyptus grandis* | Australia | T.I. Burgess & G. Pegg | DQ103554 | DQ103572 |
| *L. subglobosa* | CMM 3872* | *Jatropha curcas* | Brazil | A.R. Machado & O.L. Pereira | KF234558 | KF226721 |
| *L. thailandica* | **CPC 22755** | *Phyllanthus acidus* | Thailand | T. Trakunyingcharoen | *KM006433* | *KM006464* |
| | **CPC 22795*** | *Mangifera indica* | Thailand | T. Trakunyingcharoen | KJ193637 | KJ193681 |
| *L. theobromae* | CBS 111530 | *Leucospermum* sp. | USA: Hawaii | J.E. Taylor | EF622074 | EF622054 |
| | CBS 164.96* | Fruit along coral reef coast | Papua New Guinea | A. Aptroot | AY640255 | AY640258 |
| | **CPC 22766** | *Pinus kesiya* | Thailand | T. Trakunyingcharoen | *KM006436* | *KM006467* |
| | **CPC 22780** | *Manilkara zapota* | Thailand | T. Trakunyingcharoen | *KM006442* | *KM006473* |
| | **CPC 22798** | *Syzygium samarangense* | Thailand | T. Trakunyingcharoen | *KM006454* | *KM006485* |

**Table 1** (cont.)

| Species | Accession no.[1] | Substrate | Locality | Collector | GenBank[2] | |
|---|---|---|---|---|---|---|
| | | | | | ITS | EF1-α |
| *L. venezuelensis* | CMW 13513 | *Acacia mangium* | Venezuela | S. Mohali | DQ103549 | DQ103570 |
| *L. viticola* | CBS 128313* | *Vitis vinifera* | USA | R.D. Cartwright & W.D. Gubler | HQ288227 | HQ288269 |
| *Lasiodiplodia* sp. | **CPC 22800** | *Mangifera indica* | Thailand | T. Trakunyingcharoen | KJ193643 | KJ193687 |
| *Neofusicoccum arbuti* | CBS 116131* | *Arbutus menziesii* | USA | M. Elliot | GU251152 | GU252284 |
| *Nf. australe* | CMW 6837* | *Acacia* sp. | Australia | M.J. Wingfield | AY339262 | AY339270 |
| *Nf. luteum* | CBS 110299* | *Vitis vinifera* | Portugal | A.J.L. Phillips | AY259091 | AY573217 |
| *Nf. parvum* | CMW 9081* | *Populus nigra* | New Zealand | G.J. Samuels | AY236943 | AY236888 |
| | **CPC 22751** | *Prunus cerasoides* | Thailand | T. Trakunyingcharoen | *KM006429* | *KM006460* |
| | **CPC 22752** | *Prunus cerasoides* | Thailand | T. Trakunyingcharoen | *KM006430* | *KM006461* |
| | **CPC 22757** | *Eucalyptus obliqua* | Thailand | T. Trakunyingcharoen | *KM006435* | *KM006466* |
| *Nf. ribis* | CBS 115475* | *Ribes* sp. | USA | B. Slippers & G. Hudler | AY236935 | AY236877 |
| *Neoscytalidium hyalinum* | CBS 312.90 | *Homo sapiens* | Netherlands | R. Benne | KJ193679 | KJ193723 |
| *Ns. novaehollandiae* | CBS 122071* | *Crotalaria* sp. | Australia: Western Australia | T.I. Burgess & M.J. Wingfield | EF585540 | EF585580 |
| *Phaeobotryon cupressi* | CBS 124700* | *Cupressus sempervirens* | Iran | M.A. Aghajani | FJ919672 | FJ919661 |
| *Ph.mamane* | CBS 122980* | *Sophora chrysophylla* | USA: Hawaii | W. Gams | EU673332 | EU673298 |
| *Phyllosticta citricarpa* | CBS 111.20 | *Citrus* sp. | Australia | – | FJ538314 | FJ538372 |
| *Pseudofusicoccum adansoniae* | CBS 122055* | *Adansonia gibbosa* | Australia: Western Australia | T.I. Burgess & M.J. Wingfield | EF585523 | EF585571 |
| | **CPC 22763** | *Hevea brasiliensis* | Thailand | T. Trakunyingcharoen | KJ607148 | KJ607158 |
| | **CPC 22764** | *Hevea brasiliensis* | Thailand | T. Trakunyingcharoen | KJ607149 | KJ607159 |
| | **CPC 22765** | *Hevea brasiliensis* | Thailand | T. Trakunyingcharoen | KJ607150 | KJ607160 |
| | **CPC 22767** | *Dimocarpus longan* | Thailand | T. Trakunyingcharoen | *KM006437* | *KM006468* |
| | **CPC 22786** | *Cassia fistula* | Thailand | T. Trakunyingcharoen | *KM006446* | *KM006477* |
| | **CPC 22797** | *Senna siamea* | Thailand | T. Trakunyingcharoen | *KM006453* | *KM006484* |
| *Ps. ardesiacum* | CBS 122062* | *Adansonia gibbosa* | Australia: Western Australia | T.I. Burgess & M.J. Wingfield | EU144060 | EU144075 |
| | **CPC 22785** | *Caesalpinia pulcherrima* | Thailand | T. Trakunyingcharoen | *KM006445* | *KM006476* |
| | **CPC 22804** | *Veitchia merrillii* | Thailand | T. Trakunyingcharoen | *KM006459* | *KM006490* |
| *Ps. artocarpi* | **CPC 22796** | *Artocarpus heterophyllus* | Thailand | T. Trakunyingcharoen | *KM006452* | *KM006483* |
| *Ps. kimberleyense* | CBS 122058* | *Acacia synchronicia* | Australia: Western Australia | T.I. Burgess & M.J. Wingfield | EU144057 | EU144072 |
| *Ps. olivaceum* | CBS 124939* | *Pterocarpus angolensis* | South Africa | J. Roux | FJ888459 | FJ888437 |
| *Ps. stromaticum* | CBS 117448 | *Eucalyptus*-hybrid | Venezuela | S. Mohali | AY693974 | AY693975 |
| *Ps. violaceum* | CBS 124936* | *Pterocarpus angolensis* | South Africa | J. Mehl & J. Roux | FJ888474 | FJ888442 |

[1] CBS = CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMM = Phytopathogenic Fungi of the Universidade Federal Rural de Pernambuco; CMW = Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; CPC = Culture Collection of P.W. Crous, housed at CBS; MUCC = Murdoch University Culture Collection, Perth, Australia.
[2] ITS = internal transcribed spacers and intervening 5.8S nrDNA; EF1-α = partial translation elongation factor 1-alpha gene.

The aim of the present study was thus to identify species belonging to *Aplosporellaceae* and *Botryosphaeriaceae* collected from various plant hosts in Thailand by employing a polyphasic approach incorporating morphological, cultural and phylogenetic DNA data.

## MATERIALS AND METHODS

### *Isolates and morphology*

Both asymptomatic and symptomatic twigs and stems (associated with canker and dieback disease) were collected during February–June (2012) from various plant hosts located in Chiang Mai and Chiang Rai provinces of Thailand. The collected plant specimens included avocado (*Persea americana*), cananga (*Cananga odorata*), cassod (*Senna siamea*), Chinese juniper (*Juniperus chinensis*), coffee (*Coffea arabica*), *Eucalyptus obliqua*, fig (*Ficus racemosa*), golden shower (*Cassia fistula*), guava (*Psidium* sp.), Indian gooseberry (*Phyllanthus emblica*), longan *(Dimocarpus longan)*, mango (*Mangifera indica*), Marian plum (*Bouea burmanica*), Para rubber (*Hevea brasiliensis*), palm (*Veitchia merrillii*), peacock flower (*Caesalpinia pulcherrima*), pine (*Pinus kesiya*), rose apple (*Syzygium samarangense*), sapodilla (*Manilkara zapota*), star gooseberry (*Phyllanthus acidus*), sweet osmanthus (*Osmanthus fragrans*) and wild Himalayan cherry (*Prunus cerasoides*). Samples were incubated in moist chambers at room temperature for 7–10 d to induce sporulation. Single propagule isolations were established on 2 % Potato Dextrose Agar (PDA) and incubated at room temperature for 7 d using the techniques explained by Crous et al. (1991). Isolates of *Aplosporellaceae* and *Botryosphaeriaceae* were primarily characterised based on colony morphology, together with morphology of their asexual and sexual morphs. To induce sporulation, isolates were inoculated onto sterile pine needles and placed on 2 % water agar (PNA; Smith et al. 1996) at 25 °C under near-ultraviolet light for 14–30 d. Fungal structures were mounted in clear lactic acid and studied under a Nikon Eclipse 80i compound microscope with differential inference contrast (DIC) illumination. Thirty measurements were made of each structure, and for spores the 95 % percentiles are presented, with extremes given between brackets. The isolates used in this present study are maintained in the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands (Table 1). Reference specimens were deposited in the CBS fungarium, and nomenclature and descriptions of taxonomic novelties in MycoBank (Crous et al. 2004).

### *DNA extraction, PCR amplification and sequencing*

Genomic DNA was extracted from the 7–10-d-old mycelium growing on 2 % malt extract agar (MEA) using the UltraClean® Microbial DNA Isolation Kit (MOBIO Laboratories, Inc, Carlsbad, USA) following the manufacturer's instructions. The internal transcribed spacer (ITS) and intervening 5.8S nrRNA gene region of the nuclear rDNA were amplified using primers ITS5 and ITS4 (White et al. 1990). The partial sequences of the EF1-α gene region was amplified using primers EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell et al. 1998). However, for some isolates in the genus *Lasiodiplodia*, this region was amplified using primers EF1-688F and EF1-1251R as described by Alves et al. (2008). Master mixes for amplification followed Ismail et al. (2012). The amplifications were conducted in a thermal cycler using the following amplification conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 1 min, and a final extension step at 72 °C for 7 min.

The amplified fragments were sequenced in both directions with the same primers used for amplification, using the BigDye Terminator v. 3.1 Cycle Sequencing Kit following the manufac-

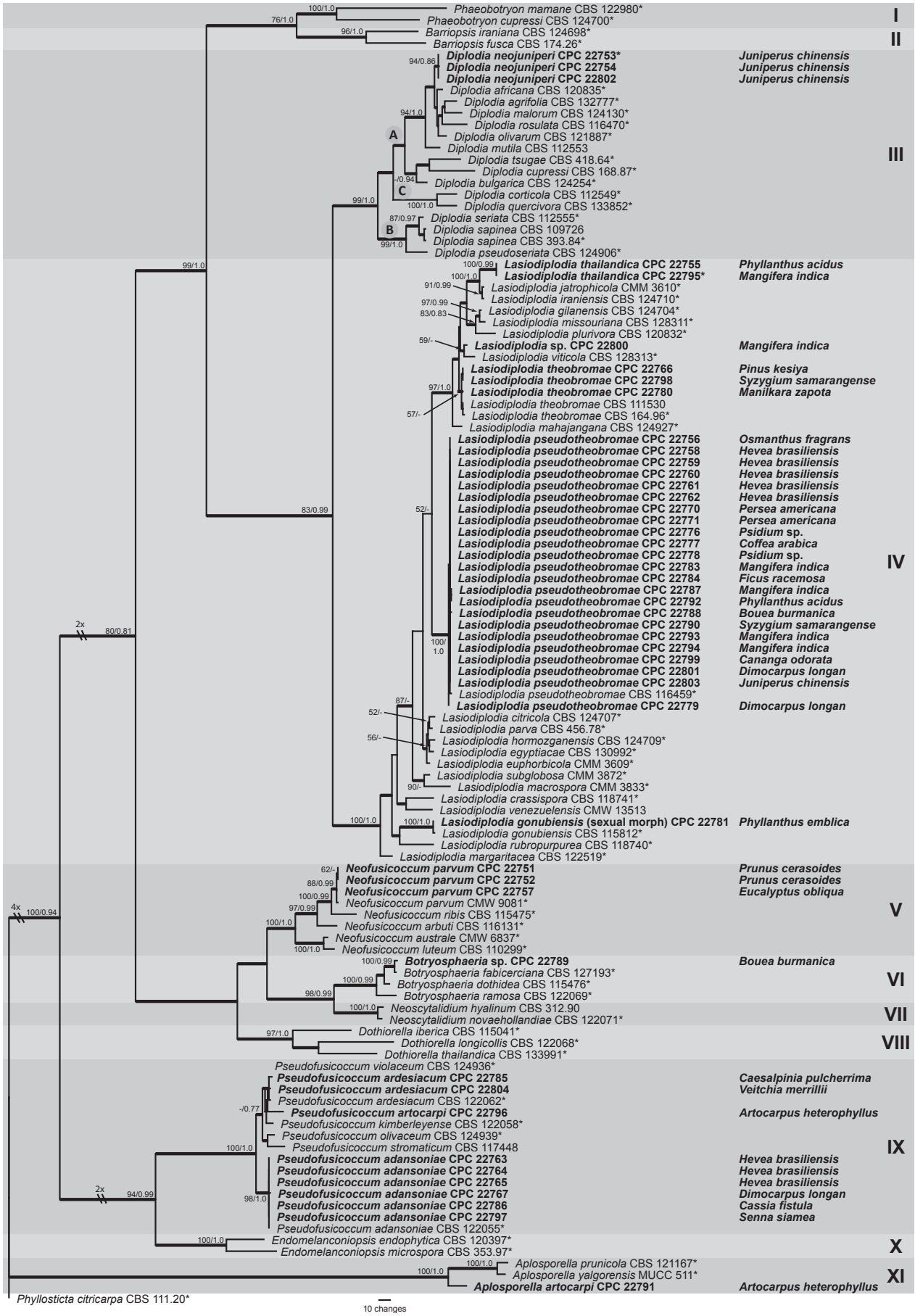


**Fig. 1** The first of 1 000 equally most parsimonious trees (TL = 2 493; CI = 0.523; RI = 0.892; RC = 0.466) resulting from a parsimony analysis of the combined ITS and EF1-α sequence alignment. The bootstrap support values (integers; to the left of the forward slash) and posterior probability values (≤ 1; to the right of the forward slash) are indicated at the nodes and the scale bar represents the number of changes. Thickened branches reflect those branches present in the strict consensus tree. Genera are indicated by different coloured blocks and provided with clade numbers in Roman numerals to the right of the tree. Species and strains from Thailand pertinent to this study are shown in **bold** and hosts from Thailand are printed in the middle of the tree, in line with the corresponding strain. The tree was rooted to *Phyllosticta citricarpa* (CBS 111.20).

turer's instructions. The sequencing reactions were run on an ABI PRISM™ 3730 DNA automated sequencer (Perkin-Elmer Applied BioSystems, Foster City, CA, USA).

### *Phylogenetic analyses*

The generated nucleotide sequences were edited, and adjustments were made manually where necessary with MEGA v. 5.1 (Tamura et al. 2011). The consensus sequences were aligned using the online version of MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/). New sequences from this study were deposited in GenBank, and were analysed together with additional sequences of species in *Botryosphaeriaceae* obtained from GenBank (Table 1). The phylogenetic analysis was performed on the combined dataset of the ITS and EF1-α regions using PAUP v. 4.0b10 (Swofford 2003) for Maximum Parsimony (MP) and MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) for Bayesian Inference (BI), respectively. Trees were rooted to *Phyllosticta citricarpa* (CBS 111.20).

The MP analysis was performed using the heuristic search option with 1 000 random stepwise additions, and tree bisection and reconnection (TBR) as branch swapping algorithm (Swofford & Begle 1993). All characters were unordered and had equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple equally parsimonious trees were saved. The robustness of the equally most parsimonious trees was calculated using 1 000 bootstrap replications (Hillis & Bull 1993). Other calculated values for parsimony included tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI).

The BI was performed by two independent runs of Markov Chain Monte Carlo (MCMC) algorithms (Larget & Simon 1999) to construct the phylogenetic tree. Four MCMC chains were run simultaneously, with heating parameter set at 0.3, under a general time-reversible (GTR) (Rodriguez et al. 1990) substitution model with rate variation of gamma-distribution (G), and proportion of invariable site (I) with a dirichlet state frequency parameters determined using MrModel Test v. 2.2 (Nylander 2004). The analyses were run for 100 000 000 generations until the average standard deviation of split frequencies came below 0.01, with trees saved every 1 000 generations. The first 25 % of saved trees were discarded as the 'burn-in' phase and the posterior probabilities (Rannala & Yang 1996) were calculated from the remaining trees.

## RESULTS

### *PCR amplification, sequencing and phylogenetic analyses*

The generated amplicons of the ITS region were ± 570 bp using the ITS5 and ITS4 primer pair. The generated amplicons of EF1-α region were ± 500 and 700 bp using the set of primers EF1-728F and EF-2, and EF1-688F and EF1-1251R, respectively. The sequences of the two amplified regions were aligned and analysed using MP and BI. New sequences in this study were deposited in GenBank as shown in Table 1 and the alignment and tree were deposited in TreeBASE.

The two datasets were congruent, and therefore combined. The alignment of the combined dataset of the ITS (628 characters; 283 unique site patterns) and EF1-α (392 characters; 344 unique site patterns) region consisted of 112 taxa with 1 020 characters including gaps, of which 361 characters were constant, 141 characters were variable and parsimony uninformative and 518 characters were parsimony informative. The heuristic search resulted in 1 000 equally most parsimonious trees with TL = 2 493, CI = 0.523, RI = 0.892, RC = 0.466 and HI = 0.477. The BI analysis lasted 2 820 000 generations and produced 5 642 trees of which 4 232 trees were sampled to produce a 50 % majority rule consensus Bayesian tree with nearly identical overall topology to the equally most parsimonious trees (Bayesian tree not shown, but posterior probability values are mapped to the parsimony tree presented in Fig. 1). The first of 1 000 equally most parsimonious trees, which showed the same overall topology, is shown in Fig. 1 with bootstrap support values and posterior probabilities indicated at the branch nodes. The parsimonious tree revealed 11 well-supported clades corresponding to established genera. Members of the *Botryosphaeriaceae* are indicated in Clades I–X. Clade I represents species of genus *Phaeobotryon*, Clade II species of *Barriopsis* and Clade III species of *Diplodia*. Clade IV is the dominant clade representing species of *Lasiodiplodia*. Clade V represents species of *Neofusicoccum*, Clade VI species of *Botryosphaeria*, Clade VII species of *Neoscytalidium* and Clade VIII species of *Dothiorella*. Clade IX represents species of *Pseudofusicoccum*, which are closely related to species in *Endomelanconiopsis* in Clade X. Clade XI represents species of *Aplosporella* (*Aplosporellaceae*), while *Phyllosticta citricarpa* (CBS 111.20) a member of *Phyllostictaceae*, was used as outgroup in this phylogenetic analysis.

The isolates obtained from Thailand clustered into six clades that included *Aplosporella*, *Diplodia*, *Fusicoccum*, *Lasiodiplodia*, *Neofusicoccum* and *Pseudofusicoccum*. The genus *Lasiodiplodia* contained several species and appeared to be the dominant group collected from Thailand in this study. New species identified in present study are described below and include *Aplosporella artocarpi* (Clade XI), *Diplodia neojuniperi* (Clade III), *Lasiodiplodia thailandica* (Clade IV) and *Pseudofusicoccum artocarpi* (Clade IX). In addition, this study is the first report of a sexual morph for *L. gonubiensis*.

### *Isolates and morphology*

Members of *Aplosporellaceae* and *Botryosphaeriaceae* obtained from Thailand clustered in six phylogenetic clades, with each clade correlating with distinct morphological features of specific genera. The isolates formed asexual structures on sterile pine needles on WA within 2–4 wk of incubation. However, no sexual morph could be induced on any of the media tested.

### *Taxonomy*

***Aplosporella artocarpi*** T. Trakunyingcharoen, L. Lombard & Crous, *sp. nov.* — MycoBank MB810167; Fig. 2

*Etymology*. The name refers to the host genus from which it was collected, *Artocarpus*.

*Conidiomata* pycnidial, semi-immersed, mostly solitary, dark brown to black, with globose base, (350–)540–550(–650) × (490–)540–600(–700) µm, outer layers composed of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region, multilocular, with (2–)4–5(–6) locules. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, subcylindrical, discrete, holoblastic, proliferating percurrently, forming 1–2 annellations near the apex, originating from the hyaline, inner conidiomatal wall, 3–5 × 2–4 µm. *Paraphyses* hyaline, cylindrical, with bluntly rounded apical cells, (13–)23–55(–60) × 2–3 µm. *Conidia* hyaline, ellipsoid to ovoid, smooth, moderately thick-walled, with granular content, aseptate, becoming pale brown before conidiomatal discharge, sometimes while still attached to the conidiogenous cells, becoming brown with rough outer surface, (17–)18–21(–22) × (9–)10–11 µm.

Culture characteristics — Colonies with white aerial mycelium on PDA, slightly fluffy, turning smokey grey with age, darker grey at the centre, sometimes mycelium turning yellowish to

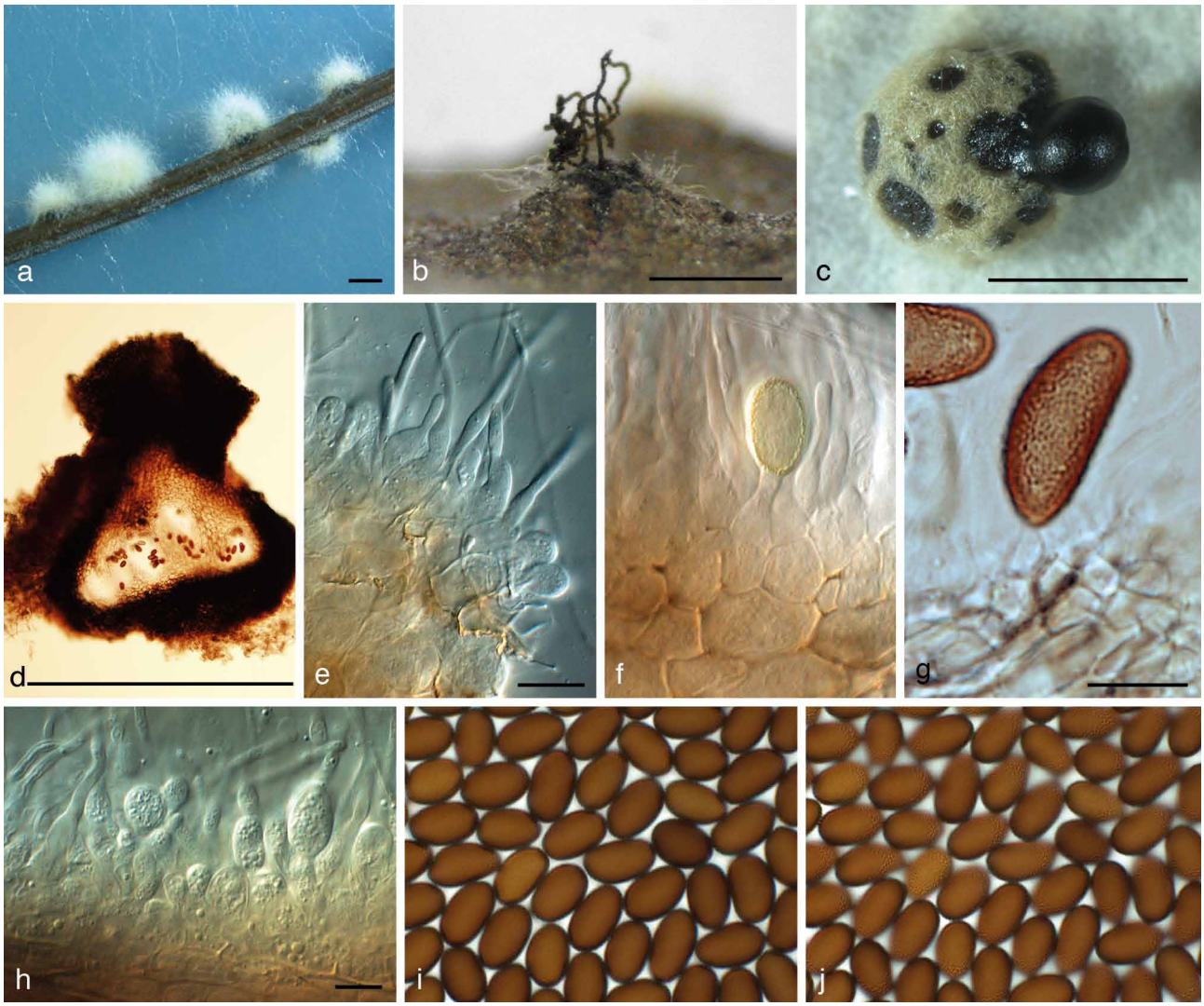


**Fig. 2** *Aplosporella artocarpi* (CBS 138651). a, b. Conidiomata sporulating on PNA; c. sporulation on PDA; d. vertical section through conidioma; e, f, h. conidiogenous cells and paraphyses; g. conidiogenous cells giving rise to conidium; i, j. brown conidia with surface ornamentation. — Scale bars: a–d = 550 µm, e–j = 10 µm.

green at the colony margin, and forming conidiomata at the colony margin after 7–10 d; colonies turn dark grey to olivaceous green in reverse.

Habitat — Asymptomatic twig of *Artocarpus heterophyllus*.

Known distribution — Chiang Mai province, Thailand.

*Material examined*. Thailand, Chiang Mai province, on twigs of *Artocarpus heterophyllus*, May 2012, *T. Trakunyingcharoen* (holotype CBS H-21931, culture ex-type CPC 22791 = CBS 138651). Additional isolates are listed in Table 1.

Notes — Although the genus *Aplosporella* has previously been treated as a member of the *Botryosphaeriaceae* (Damm et al. 2007b), it was recently placed in its own family *Aplosporellaceae* (Slippers et al. 2013). *Aplosporella artocarpi* has been introduced as new species based on its distinct phylogenetic position and morphological features. Although conidial dimensions of *A. artocarpi* (17–)18–21(–22) × (9–)10–11 µm overlap with those of *A. prunicola* (17–)19–22(–25) × (9–)10–12(–18) µm and *A. yalgorensis* (16–)18–22(–26) × (7–)8–13(–14) µm (Damm et al. 2007b, Taylor et al. 2009), conidia of *A. artocarpi* are shorter and narrower than conidia of these two species. In addition, conidia of *A. artocarpi* are narrower than those of *A. embeliae* (18–22 × 12–16 µm), wider than *A. subhyalina* (18–22 × 4–6 µm) and longer than *A. beaumontiana* (13–20 × 10–11.5 µm) and *A. clerodendri* (12–16 × 8–10 µm) (Pande & Rao 1995). Nothing is presently known about the host specificity of species of *Aplosporella*, and thus far very few species are known from culture.

***Diplodia neojuniperi*** T. Trakunyingcharoen, L. Lombard & Crous, *sp. nov.* — MycoBank MB810168; Fig. 3

*Etymology*. The name refers to its morphological similarity to *D. juniperi*.

*Conidiomata* pycnidial, immersed, dark brown to black, with globose base, solitary, unilocular, 230–330 × 200–320 µm, outer layers composed of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Ostiole* central, circular, up to 50 µm diam, papillate, with neck up to 20 µm tall. *Conidiophores* (when present) hyaline, cylindrical, 0–1-septate, 8–10 × 2.5–3 µm, mostly reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, thin-walled, discrete, cylindrical to ampulliform, holoblastic, proliferating to form 1–2 annellations or proliferating at the same level with visible periclinal thickening, generated from the hyaline inner wall of conidiomata, 9–12 × 2.5–3 µm. *Paraphyses* absent. *Conidia* hyaline, ellipsoid, unicellular, with granular content, 1 µm thick-walled, becoming pale brown with single median septum after discharge from the pycnidia, but rarely observed, (17–)18–21(–22) × (9–)10–11 µm.

Culture characteristics — Colonies forming two distinct zones on PDA. The first with grey mycelium, moderately dense and fluffy at the centre, while olivaceous grey mycelium flatten-

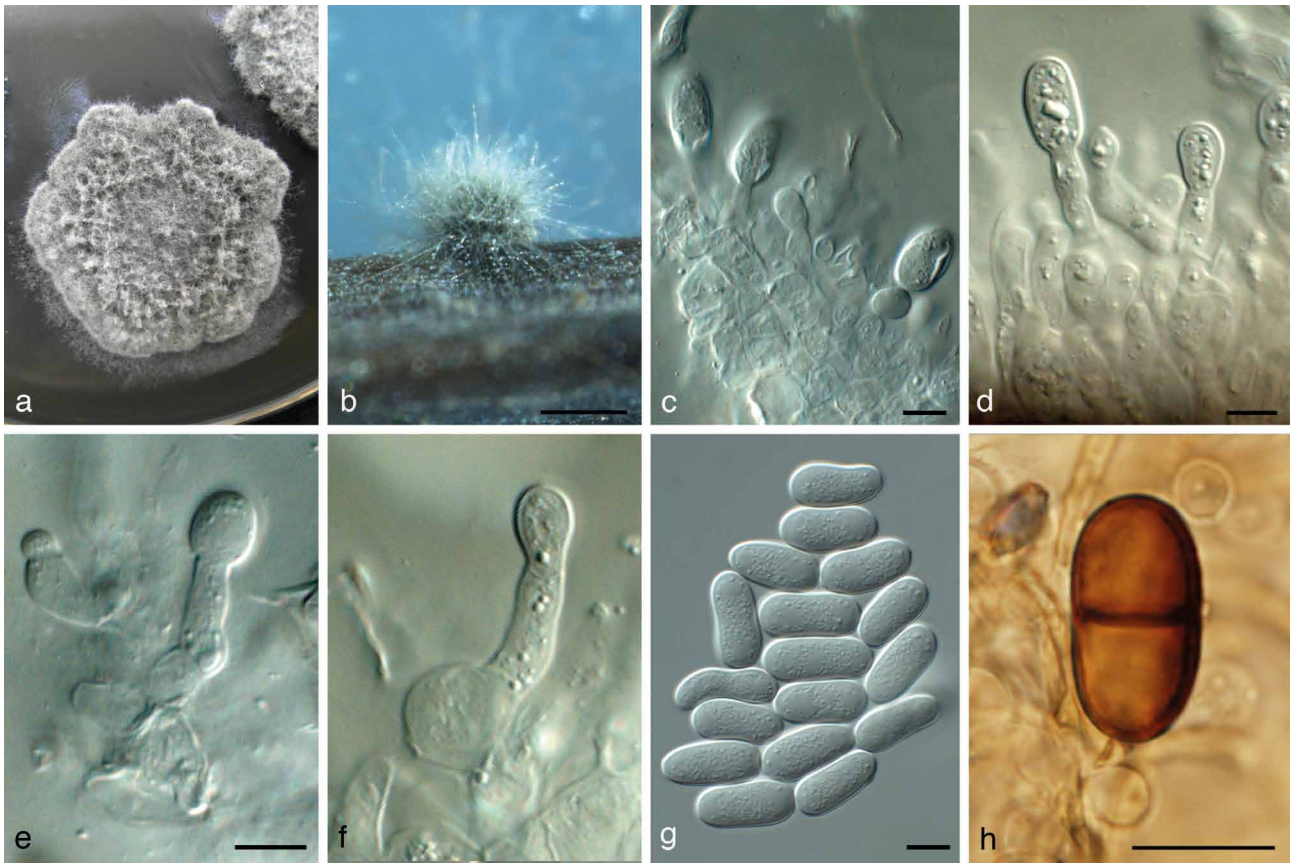


**Fig. 3** *Diplodia neojuniperi* (CBS 138652). a. Colony sporulating on MEA; b. colony sporulating on PNA; c–f. conidiogenous cells giving rise to conidia; g. hyaline conidia; h. mature, 1-septate, brown conidium. — Scale bars: b = 300 µm, all others = 10 µm.

ing at the margin, erose or dentate, greenish olivaceous to black olivaceous in reverse after 7 d.

Habitat — Associated with leaf blight symptoms on *Juniperus chinensis.*

Known distribution — Chiang Mai province, Thailand.

*Material examined*. Thailand, Chiang Mai province, on leaf of *Juniperus chinensis*, Feb. 2012, *T. Trakunyingcharoen* (holotype CBS H-21932, culture ex-type CPC 22753 = CBS 138652). Additional isolates are listed in Table 1.

Notes — Species of *Diplodia* are presently classified in three subclades (A–C) based on the molecular data and morphological characteristics (Phillips et al. 2013). Subclade A is a dominant group and consists of a number of species which include *D. africana*, *D. agrifolia*, *D. bulgarica*, *D. cupressi*, *D. malorum*, *D. mutila*, *D. olivarum*, *D. rosulata*, *D. tsugae* and now also *D. neojuniperi*. These species have hyaline conidia, which become pigmented and 1-septate after conidial discharge. Species of subclade B include *D. sapinea* and *D. seriata*. They have hyaline conidia, which turn pigmented at an early stage while still in their conidiomata, sometimes even while still attached to their conidiogenous cells. Although members of subclade C (*D. corticola* and *D. quercivora*) have similar morphological characteristics to those in subclade A, their conidia are much larger (Table 2).

To distinguish *D. neojuniperi* from other species within subclade A, it needs to be compared to its closest neighbours, *D. africana* (based on MP analysis) and *D. mutila* (based on the BI analysis; data not shown). According to its conidial morphology, conidia of *D. neojuniperi* are much smaller than those of *D. africana* and *D. mutila* (Table 2). Saccardo (1884) reported *D. juniperi* from *Juniperus* in Europe (conidia 18–20 × 8–10 µm). However, an examination of the type specimen (BR-Myc 148292,76) by Alves et al. (2006) failed to reveal any *Diplodia* material, and all fresh collections only rendered *D. cupressi* (conidia 21.5–30.5 × 12–16 µm). For this reason, as well as its geographic separa-

**Table 2** A comparison of conidial morphology of *Diplodia* spp.

| Species | Group sensu Phillips et al. (2013) | Conidial dimensions (µm) | Reference |
|---|---|---|---|
| *D. africana* | A | (17–)25.5–33(–34) × (10–)12–14(–15) | Damm et al. (2007a) |
| *D. agrifolia* | A | (21.5–)27–36.5 × (12–)14.5–18 | Lynch et al. (2013) |
| *D. bulgarica* | A | (22.5–)24–27(–28) × (14.5–)15.5–18(–18.5) | Phillips et al. (2012) |
| *D. corticola* | C | (23.5–)26–34.5(–46) × (9–)12–16(–18.5) | Alves et al. (2004) |
| *D. cupressi* | A | (21.5–)23.5–28.5(–30.5) × (12–)13.5–15(–16) | Alves et al. (2006) |
| *D. malorum* | A | (24–)26–32(–36) × (12–)13–17.5(–18.5) | Phillips et al. (2012) |
| *D. mutila* | A | (23.5–)24.5–27(–27.5) × (12.5–)13–14(–14.5) | Montagne (1834) |
| *D. neojuniperi* | A | (17–)18–21(–22) × (9–)10–11 | Present study |
| *D. olivarum* | A | (21.5–)22–27.5(–28.5) × (10–)11–13.5(–14.5) | Lazzizera et al. (2008) |
| *D. quercivora* | C | (22.75–)28.14(–30.41) × (11.32–)13.08(–14.36) | Linaldeddu et al. (2013) |
| *D. rosulata* | A | (21–)25–32(–36) × (10–)11–17.5(–19.5) | Gure et al. (2005) |
| *D. sapinea* | B | (25.5–)30.5–52.5(–54) × (10–)12.5–20(–21) | Fuckel (1870) |
| *D. seriata* | B | (21.5–)22–27(–28) × (11–)11.5–14.5(–15.5) | de Notaris (1845) |
| *D. tsugae* | A | 36–41 × 18–22 | Phillips et al. (2012) |


**Fig. 4** *Lasiodiplodia gonubiensis* (CBS 138654). a. Ascomata imbedded in host tissue; b, c. section through ascomata; d, e; asci. f–j. hyaline, young ascospores, that become brown and septate with age; k. conidiomata forming on PNA; l, m. conidiogenous cells giving rise to conidia; n. conidia. — Scale bars: a, b = 450 µm, c = 225 µm, all others = 10 µm.

tion and wider conidial dimensions, the material from Thailand is described here as a novel species on juniper.

***Lasiodiplodia gonubiensis*** Pavlic, Slippers & MJ. Wingf., Stud. Mycol. 50: 318. 2004. — Fig. 4

*Ascomata* perithecial, immersed under bark, sometimes semi-immersed, solitary to aggregated, dark brown to black, unilocular, with globose base, (400–)530–600(–750) × (330–)400–500(–570) µm, outer layers composed of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Ostiole* central, circular, up to 80 µm diam, neck papillate, (50–)100–120(–150) µm tall. *Pseudoparaphyses* hyaline, cylindrical, smooth, thin-walled, multi-septate, 73–125 × 3–4 µm. *Asci* hyaline, bitunicate with thick endotunica and well-developed apical chamber, clavate and stalked, sessile, originating from the inner hyaline wall of ascoma, (120–)150–180(–200) × (22.5–)27.5–30(–32.5) µm, containing (4–)6–8 ascospores. *Ascospores* hyaline, broadly fusiform to rhomboid to limoniform, moderately thick-walled with granular content, widest in the middle, ascospores with hyaline apiculus at both or either end, rarely becoming 1–2-septate with age, ascospores turn pale brown, 1–2-septate within ascoma or shortly after discharge, (32.5–)35–37.5(–40) × (16–)17.5–20(–25) µm. The asexual morph was described in full by Pavlic et al. (2004).

Culture characteristics — Colonies with white, fluffy aerial mycelium on PDA, becoming smoky-grey to olivaceous-grey, moderately dense at the centre of colony, a part of the colony forming wool-like aerial mycelium; reverse becoming greenish grey to olivaceous-grey after 7 d. Cultures remain sterile.

**Table 3** Morphological comparison of *Botryosphaeriaceae* with dark ascospores.

| Species | Ascospores | | | Reference |
|---|---|---|---|---|
| | Septation | Apiculus | Dimensions (µm) | |
| *Barriopsis fusca* | aseptate | absent | (30–)31–36.5(–38.5) × (15.5–)16–18.5(–21) | Phillips et al. (2008) |
| *Dothiorella iberica* | 1-septate | absent | (17.5–)22.5–23.5(–29) × (8.5–)10–10.5(–12.5) | Phillips et al. (2005) |
| *Lasiodiplodia gonubiensis* | rarely 1–2-septate | present | (32.5–)35–37.5(–40) × (16–)17.5–20(–25) | Present study |
| *Phaeobotryon mamane* | 2-septate | present | (30–)37–40(–45) × (11–)13–15(–16) | Phillips et al. (2008) |
| *Spencermartinsia viticola* | 1-septate | present | (19–)22.5–23.5(–27) × (8.5–)10.5–11(–14.5) | Saccardo (1880) |
| *Sphaeropsis visci* | aseptate | present | (27.5–)31–37.5(–38.5) × (14.5–)15–19(–19.5) | Phillips et al. (2008) |

Habitat — Symptomless twigs of *Phyllanthus emblica.*

Known distribution — The reserved forest of Aomkoi district, Chiang Mai province, Thailand.

*Material examined*. Thailand, Chiang Mai province, on twigs of *Phyllanthus emblica*, Apr. 2012, *T. Trakunyingcharoen* (CBS H-21934, culture CPC 22781 = CBS 138654). Additional isolates are listed in Table 1.

Notes — Ascospores of *L. gonubiensis* are normally aseptate, and rarely turn pale brown and 1–2-septate when mature like *Barriopsis*, *Phaeobotryon* and *Sphaeropsis*. In contrast, ascospores of *L. gonubiensis* are distinct from the sexual morphs of *Dothiorella* and *Spencermartinsia*, which are 1-septate. Ascospores of *L. gonubiensis* have a terminal apiculus, which is not found in *Barriopsis*. Thus ascospores of *L. gonubiensis* are morphologically distinct from other sexual morphs in the *Botryosphaeriaceae* based on its size, the presence of a terminal apiculus, and by becoming pigmented and septate upon ascospore release (Table 3).

***Lasiodiplodia thailandica*** T. Trakunyingcharoen, L. Lombard & Crous, *sp. nov.* — MycoBank MB810169; Fig. 5

*Etymology*. Name refers to the country where this fungus was collected, Thailand.

*Conidiomata* pycnidial, semi-immersed, solitary, rarely aggregated, dark brown to black, unilocular, with globose base, 310–330 × 300–370 µm, outer layers composed of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region, hyphal hairs brown, septate, 60–150 × 5–6 µm, with rounded tips covering the outer wall of fruiting body. *Ostiole* central, circular, 40–60 µm diam; conidiomata papillate, neck 60–110 µm tall. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, thin-walled, discrete, cylindrical, holoblastic, proliferating percurrently from hyaline inner conidiomatal wall, 8–9 × 2–4 µm. *Paraphyses* hyaline, smooth, thin-walled, cylindrical, originating from the hyaline inner cells of pycnidial wall, the basal cells often slightly swollen, the apical cells with end-rounded tip, 1–3-septate, 25–51 × 1–1.5 µm. *Conidia* initially hyaline, wall 1.2–1.5 µm thick, ellipsoid, with granular content, unicellular, (20–)22–25(–26)

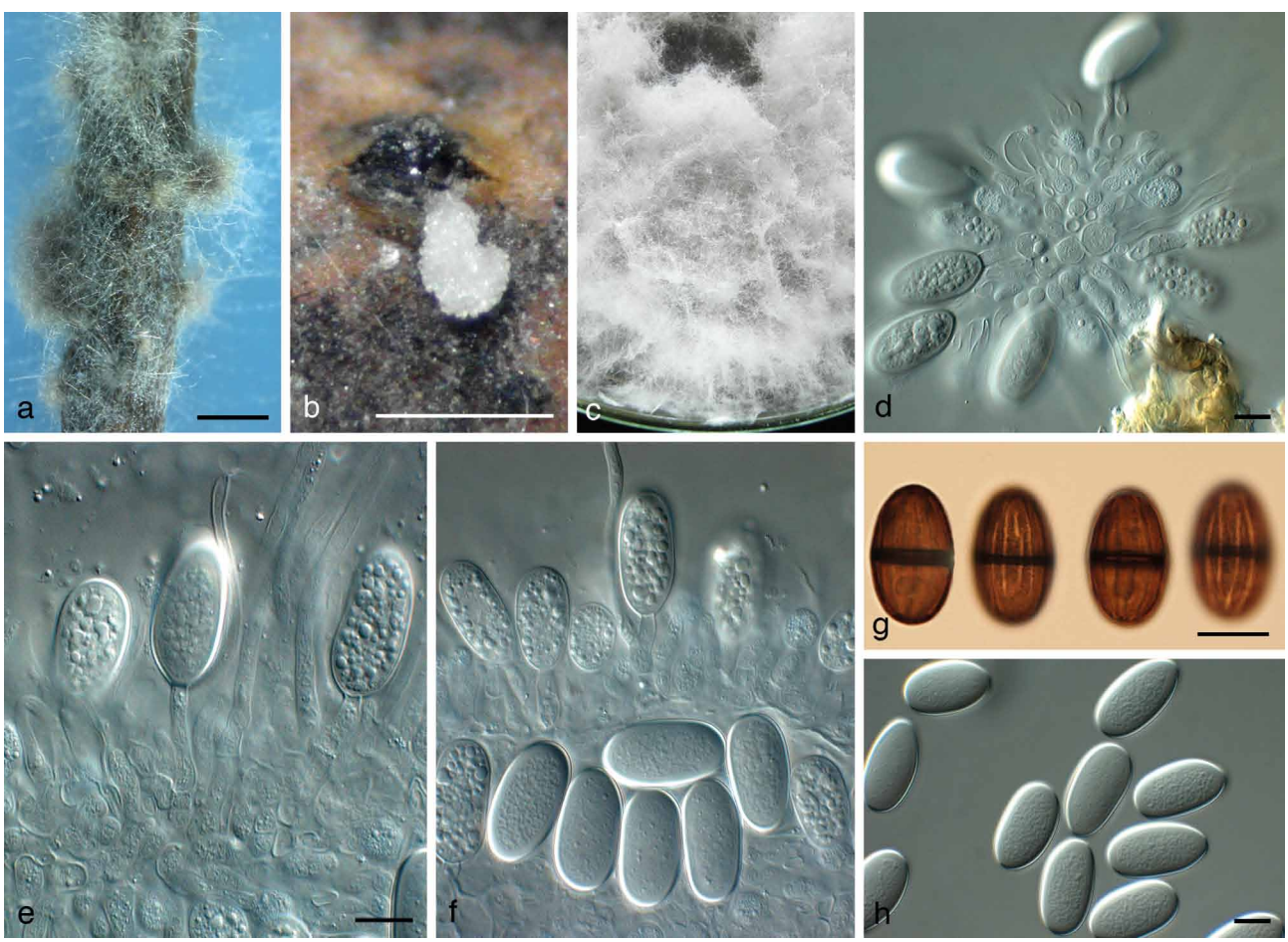


**Fig. 5** *Lasiodiplodia thailandica* (CBS 138653). a, b. Colony sporulating on PNA; b. fluffy aerial mycelium on PDA; d–f. conidiogenous cells giving rise to conidia; g. brown, 1-septate conidia; h. young, hyaline conidia. — Scale bars: a, b = 300 µm, all others = 10 µm.

**Table 4** A morphological comparison of *Lasiodiplodia* spp.

| Species | Conidial dimensions (µm) | Paraphyses | | Reference |
|---|---|---|---|---|
| | | Septation | Size (µm) | |
| *L. citricola* | (20–)22–27(–31) × (10.9–)12–17(–19) | 1–5-septate | 125 × 3–4 | Abdollahzadeh et al. (2010) |
| *L. crassispora* | 27–30(–33) × 14–17 | septate | 45.7 × 2.7 | Burgess et al. (2006) |
| *L. egyptiacae* | 20–24 × 11–12 | aseptate | 57 × 2–3 | Ismail et al. (2012) |
| *L. euphorbicola* | 15–23 × 9–12 | septate | 76 × 2–4 | Machado et al. (2014) |
| *L. gilanensis* | (25.2–)28–35(–38.8) × (14.4–)15–18(–19) | 1–3-septate | 95 × 2–4 | Abdollahzadeh et al. (2010) |
| *L. gonubiensis* | (28–)32–36(–39) × (14–)16–18.5(–21) | aseptate | 38.1 × 2.3 | Pavlic et al. (2004) |
| *L. hormozganensis* | (15.3–)18–24(–25.2) × 11–14 | 1–7-septate | 83 × 2–4 | Abdollahzadeh et al. (2010) |
| *L. iraniensis* | (15.3–)17–23(–29.7) × 11–14 | 1–6-septate | 127 × 2–4 | Abdollahzadeh et al. (2010) |
| *L. jatrophicola* | 22–26 × 14–17 | 0(–1)-septate | 70 × 3 | Machado et al. (2014) |
| *L. macrospora* | 28–35 × 15–17 | septate | 105 × 3–4 | Machado et al. (2014) |
| *L. mahajangana* | (13.5–)15.5–19(–21.5) × (10–)11.5–13(–14) | aseptate | 43 × 3 | Begoude et al. (2010) |
| *L. margaritacea* | (12–)14–17(–19) × (10–)11–12(–12.5) | 1–2-septate | 37.1 × 2.2 | Pavlic et al. (2008) |
| *L. missouriana* | (16.1–)17.4–19.6(–21) × (8.1–)8.9–10.6(–11.8) | aseptate | 55 × 2–3 | Urbez-Torres et al. (2012) |
| *L. paraphysaria* | 30–32 × 15–16 | 1-septate | 90–100 × 3 | Saccardo & Sydow (1899) |
| *L. parva* | (15.5–)16–23.5(–24.5) × (10–)10.5–13(–14.5) | septate | 105 × 3–4 | Alves et al. (2008) |
| *L. plurivora* | (22–)26.5–32.5(–35) × (13–)14.5–17(–18.5) | 1–6-septate | 130 × 2–5 | Damm et al. (2007a) |
| *L. pseudotheobromae* | (22.5–)23.5–32(–33) × (13.5–)14–18(–20) | mostly aseptate | 58 × 3–4 | Alves et al. (2008) |
| *L. ricini* | 16–19 × 10–11 | 1-septate | 25–35 × 2 | Saccardo (1915) |
| *L. rubropurpurea* | 24–33 × 13–17 | aseptate | 42.4 × 2.6 | Burgess et al. (2006) |
| *L. subglobosa* | 16–23 × 11–17 | aseptate | 41 × 2–3 | Machado et al. (2014) |
| *L. thailandica* | (20–)22–25(–26) × (12–)13–15(–16) | 1–3-septate | 51 × 1–1.5 | Present study |
| *L. theobromae* | (19–)21–31(–32.5) × (12–)13–15.5(–18.5) | septate | 55 × 3–4 | Alves et al. (2008) |
| *L. thomasiana* | 28–30 × 11–12 | – | 80–90 × 1.5 | Saccardo & Trotter (1913) |
| *L. venezuelensis* | 26–33 × 12–15 | septate | 28.3 × 3.5 | Burgess et al. (2006) |
| *L. viticola* | (16.8–)18.2–20.5(–22.9) × (7.9–)8.8–10.1(–10.7) | aseptate | 60 × 2–3 | Urbez-Torres et al. (2012) |

× (12–)13–15(–16) µm, a few conidia turning pale brown with a single median septum and longitudinal striations after discharge from the pycnidia, but most of the discharged conidia remain hyaline.

Culture characteristics — Colonies with white fluffy mycelium on PDA, slightly mycelium dense and flattening at the centre, mycelium turning smoky-grey to olivaceous-grey with age, mycelium turning greenish olivaceous to black-olivaceous in reverse after 7 d.

Habitat — Symptomless twigs of *Mangifera indica*.

Known distribution — Chiang Mai province, Thailand.

*Materials examined*. Thailand, Chiang Mai province, on twigs of *Mangifera indica*, May 2012, *T. Trakunyingcharoen* (holotype CBS-H 21933, culture ex-type CPC 22795 = CBS 138760); on petiole of *Phyllanthus acidus*, Feb. 2012, *T. Trakunyingcharoen*, CBS 138653 = CPC 22755. Additional isolates are listed in Table 1.

Notes — Conidia of *L. thailandica* are much smaller than conidia of *L. crassispora*, *L. gilanensis*, *L. gonubiensis*, *L. macrospora*, *L. paraphysaria*, *L. plurivora*, *L. pseudotheobromae*, *L. rubropurpurea*, *L. theobromae*, *L. thomasiana* and *L. venezuelensis*. However, conidia of *L. thailandica* are again larger than those of *L. euphorbicola*, *L. hormozganensis*, *L. iraniensis*, *L. mahajangana*, *L. margaritacea*, *L. missouriana*, *L. parva*, *L. ricini*, *L. subglobosa* and *L. viticola*. Although the conidial size of *L. thailandica* shows some overlap with *L. citricola*, *L. egyptiacae* and *L. jatrophicola*, these taxa are distinct based on the characteristics of their paraphyses. Paraphyses of *L. thailandica* are much shorter and narrower than those of the latter three species. *Lasiodiplodia thailandica* is phylogenetically closely related to *L. iraniensis* and *L. jatrophicola*. However, these species differ morphologically based on the dimensions of their conidia and paraphyses (Table 4). Furthermore, only some conidia of *L. thailandica* become pigmented after conidial discharge, which again separates it from most other *Lasiodiplodia* species.

***Pseudofusicoccum artocarpi*** T. Trakunyingcharoen, L. Lombard & Crous, *sp. nov.* — MycoBank MB810170; Fig. 6

*Etymology*. The name refers to the host genus from which it was collected, *Artocarpus*.

*Conidiomata* pycnidial, semi-immersed, solitary to aggregated, mostly aggregated, dark brown, unilocular, rarely multilocular, with globose base, (350–)400–520(–550) × (130–)170–280(–360) µm, covered by pale brown, septate hyphal hairs, that turn brown with age, (60–)80–180(–250) × 2–3(–4) µm; outer layers composed of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region, 5–6 cells thick. *Ostiole* central, circular, rarely with 2 ostioles, ostiole 40–50 µm diam; conidiomata papillate, neck length 80–100(–140) µm tall. *Conidiophores* hyaline, cylindrical, 9–11 × 2 µm, 0–2-septate. *Conidiogenous cells* hyaline, smooth, thin-walled, discrete, cylindrical, holoblastic, proliferating percurrently near apex, formed from the hyaline inner wall of the conidiomata, 8–11(–13) × (2–)3–4 µm. *Paraphyses* absent. *Conidia* hyaline, bacilliform to ellipsoid, straight to slightly curved, both apex and base blunt to broadly round, moderately thick-walled, with

**Table 5** A comparison of conidial dimensions of *Pseudofusicoccum* spp.

| Species | Conidial dimensions (µm) | Reference |
|---|---|---|
| *P. adansoniae* | (19–)21–24(–26) × (3.5–)4.5–6(–6.5) | Pavlic et al. (2008) |
| *P. ardesiacum* | (17.5–)21–29(–32) × (6.3–)7–8(–9) | Pavlic et al. (2008) |
| *P. artocarpi* | (33–)34–43(–46) × 7–8(–9) | Present study |
| *P. kimberleyense* | (24–)28–33(–34) × (6.5–)7–8(–8.5) | Pavlic et al. (2008) |
| *P. olivaceum* | (17.9–)19.9–25.7(–30.4) × (5.9–)6.3–7.7(–8.9) | Mehl et al. (2011) |
| *P. stromaticum* | (19–)20–23(–24) × (4–)5–6 | Mohali et al. (2006) |
| *P. violaceum* | (26.5–)29.8–36.1(–39.6) × (8.0–)8.7–10.3(–11.6) | Mehl et al. (2011) |

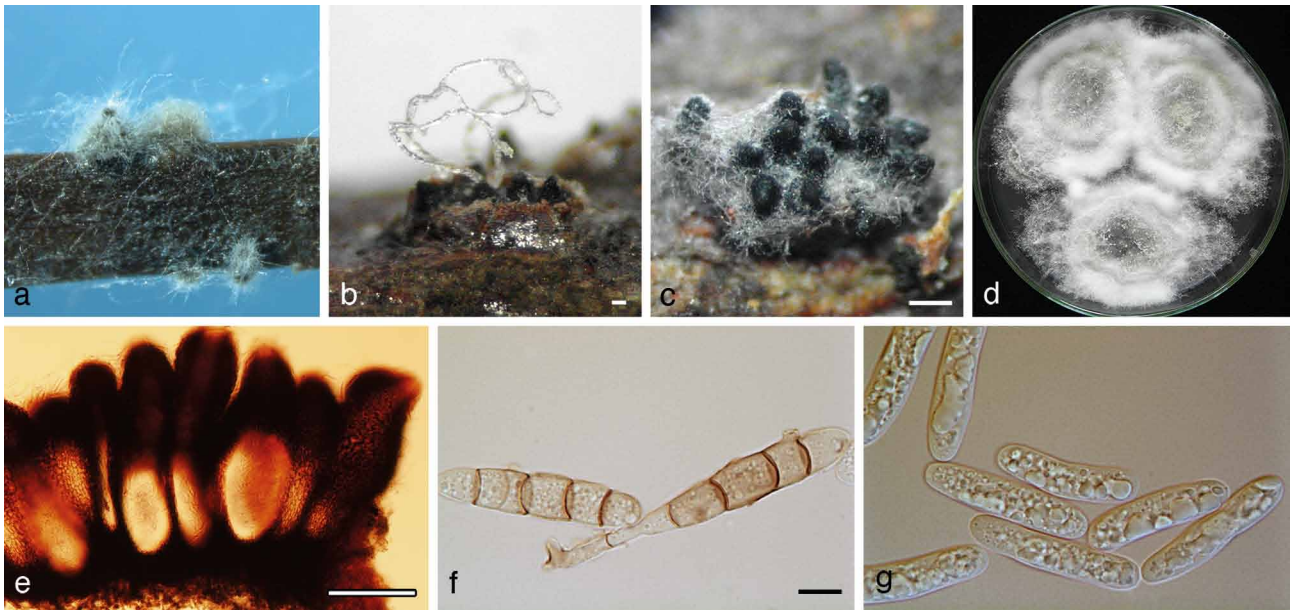


**Fig. 6** *Pseudofusicoccum artocarpi* (CBS 138655). a. Sporulation on PNA; b, c. aggregated conidiomata on host tissue; d. colony on MEA with fluffy aerial mycelium; e. section through conidiomata; f. conidia becoming brown and septate with age; g. young conidia. — Scale bars: b, c, e = 250 µm, f, g = 10 µm.

granular content, aseptate, (33–)34–43(–46) × 7–8(–9) µm, becoming pale brown and (1–)3–5(–7)-septate with age, sometimes conidia turn pale brown and septate while still attached to the conidiogenous cells.

Culture characteristics — Colonies with white, cottony aerial mycelium on PDA, moderately dense and flattening at the centre, becoming smoky-grey with age, and turning olivaceous-grey in reverse after 7 d.

Habitat — Asymptomatic twig of *Artocarpus heterophyllus.*

Known distribution — Chiang Mai province, Thailand.

*Material examined*. Thailand, Chiang Mai province, on twigs of *Artocarpus heterophyllus*, May 2012, *T. Trakunyingcharoen* (holotype CBS H-21935, culture ex-type CPC 22796 = CBS 138655). Additional isolates are listed in Table 1.

Notes — Conidia of *P. artocarpi* are clearly longer than those of other *Pseudofusicoccum* species, with conidia becoming pale brown and up to 7-septate with age (Table 5). These morphological characters are rather typical, and can easily be used to distinguish *P. artocarpi* from other *Pseudofusicoccum* species (Pavlic et al. 2008).

## DISCUSSION

Schoch et al. (2006) introduced the order *Botryosphaeriales* with the *Botryosphaeriaceae* as a single family within the order. Based on subsequent research, however, six families are presently recognised within *Botryosphaeriales*, namely *Aplosporellaceae*, *Botryosphaeriaceae*, *Melanopsaceae*, *Phyllostictaceae*, *Planistromellaceae* and *Saccharataceae* (Liu et al. 2012, Minnis et al. 2012, Slippers et al. 2013, Wikee et al. 2013). In the present study, species of *Aplosporellaceae* and *Botryosphaeriaceae* obtained from various host plants in Thailand were identified as corresponding to the genera *Aplosporella*, *Botryosphaeria*, *Diplodia*, *Lasiodiplodia*, *Neofusicoccum* and *Pseudofusicoccum* based on morphology and DNA phylogeny.

Although members of these genera are commonly encountered, not much is known about the host specificity and relative importance of the majority of species that have been described to date. Species of *Diplodia* represent important pathogens that can cause blight, canker, dieback and rot diseases on numerous host plants (Burgess et al. 2004, Lazzizera et al. 2008, Linaldeddu et al. 2011). Several *Diplodia* species have been reported to be associated with *Cupressus* and *Juniperus*, including *D. cupressi* (Alves et al. 2006) and *D. mutila* (Tisserat et al. 1988, Flynn & Gleason 1993). In the present study an undescribed *Diplodia* species morphologically similar to *D. juniper* was isolated from *Juniperus chinensis* in Thailand, and described here as *D. neojuniperi*. The genus *Lasiodiplodia* represents one of the most well-known genera in the *Botryosphaeriaceae*, with species recorded on a broad host range in tropical and subtropical regions (Punithalingam 1976). Accordingly, isolates of *Lasiodiplodia* spp. appeared to represent the most dominant clade, and have the widest distribution and host range of all isolates collected in Thailand. Species of *Lasiodiplodia* obtained from Thailand were identified as *L. pseudotheobromae*, *L. theobromae* and *L. viticola*. Furthermore, *L. thailandica* was introduced as novel taxon, and the sexual morph of *L. gonubiensis* was also reported for the first time. *Lasiodiplodia gonubiensis* was first introduced by Pavlic et al. (2004) for endophytic isolates in leaves and branches of native *Syzygium cordatum* from South Africa. The collection of its sexual morph in Thailand is not totally unexpected, as it seems that generally species of *Lasiodiplodia* have wide geographical distributions (Phillips et al. 2013).

The genus *Neofusicoccum* was first introduced by Crous et al. (2006), and includes many species that are important pathogens causing several plant diseases globally (Slippers et al. 2005, de Oliveira Costa et al. 2010, Thomidis et al. 2011, Ni et al. 2012). Although some species such as *N. arbuti* and *N. protearum* appear to be largely host-specific (Phillips et al. 2013), most species of the genus have wide host ranges and geographical distributions. The genus *Pseudofusicoccum* was introduced by Crous et al. (2006) for species resembling *Fusicoccum* (= *Botryosphaeria*), but being distinct by having a persistent mucous sheath surrounding their conidia. Although Pavlic et al. (2008) suggested that all species of *Pseudofusicoccum* could be native to Australia, species such as *P. olivaceum* and *P. violaceum* were introduced on *Pterocarpus angolensis* native to South Africa, and appear to represent the first *Pseudofusicoccum* spp. not native to Australia (Mehl et al. 2011). In the present study *P. adansoniae* and *P. ardesiacum* were also collected from native and non-native plants of Thailand, and this is the first report of these species in this region. *Pseudofusicoccum*

*adansoniae* was obtained from *Senna siamea*, *Cassia fistula*, *Dimocarpus longan* and *Hevea brasiliensis*, while *P. ardesiacum* was isolated from *Veitchia merrillii* and *Caesalpinia pulcherrima*. Furthermore, a seventh species of *Pseudofusicoccum* was newly described here as *P. artocarpi* from *Artocarpus heterophyllus*. Conidia of *P. artocarpi* are much longer than any of the presently known species, and also turn pale brown and multiseptate with age.

*Aplosporella* was previously included as a member of the *Botryosphaeriaceae* based on the molecular analyses conducted by Damm et al. (2007b). It was only recently excluded from this family and allocated to the *Aplosporellaceae* (Slippers et al. 2013). Historically, species of *Aplosporella* have mostly been described based on their host occurrence (Damm et al. 2007b). In the present study, *A. artocarpi* was introduced as a novel species from *Artocarpus heterophyllus* in Thailand. No species of *Aplosporella* have thus far been described from *Artocarpus*, and *A. artocarpi* is also distinct from all taxa presently known from their DNA sequence data. However, the genus is clearly under-represented, and many more collections would be required in an attempt to understand its host specificity and potential pathogenicity.

In general, species of *Botryosphaeriaceae* have cosmopolitan distributions and broad host ranges. They are commonly encountered as endophytes and opportunistic pathogens, causing a range of important plant diseases leading to economic losses in many regions of the world (Slippers & Wingfield 2007). Very little has been known about botryosphaeriaceous fungi in Thailand until the recent study of Liu et al. (2012). Likewise, the present study adds four new species and three new records from native and non-native plant hosts in Thailand as belonging to the *Aplosporellaceae* and *Botryosphaeriaceae*. Further studies on the ecology, epidemiology, distribution and pathogenicity of these taxa is now urgently required to provide a better understanding about the importance and potential impact that these fungi may have on woody hosts in Thailand.

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